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MATRIX METALLOPROTEINASE AND TISSUE INHIBITOR OF METALLOPROTEINASE ACTIVITY IN SYNOVIAL FLUID FROM HORSES WITH POST-TRAUMATIC OSTEOARTHRITIS

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Purpose: Matrix metalloproteinases (MMPs) are enzymes that are responsible for degradation of the extracellular matrix of articular cartilage. Tissue inhibitors of metalloproteinases (TIMPs) are natural inhibitors that bind 1:1 with MMPs blocking their actions. In general, MMP activity has been shown to increase when cartilage damage is present, whereas TIMP activity is variable. The objective of the current study was to compare MMP and TIMP concentrations and their ratios in synovial fluid (SF) from horses with normal joints (controls) and those with post-traumatic osteoarthritis (PTOA). Our hypothesis was that MMP concentrations would be higher and TIMP concentrations would be lower in joints with PTOA compared to controls, resulting in higher MMP to TIMP ratios in injured horses. If so, this would account for less regulation of MMP activity in injured joints, potentiating further articular cartilage damage.
Methods: SF was taken from the metacarpophalangeal ($n = 11$) and/or carpal ($n = 10$) joints from 19 horses (age range 2-14 years) that had arthroscopic evidence of PTOA and from the metacarpophalangeal ($n = 10$) and/or carpal ($n = 4$) joints from 12 control horses (age range 4-9 years) with radiographically and clinically normal joints. SF was aseptically collected by needle arthrocentesis without lavage, centrifuged, and decanted. Commercially available multiplex assays (R&D Systems, Minneapolis, MN) were used to measure MMP -1, -2, -3, -9, and -13 (Human MMP Magnetic Luminex Performance Assay) and TIMP -1, -2, -3, and -4 (Human TIMP Magnetic Luminex Performance Assay 4-plex kit) on a Luminex xMap technology based multiplex array system (Bio-Plex® 200 system, Bio-Rad Laboratories, Hercules, CA). SF was digested with hyluronidase and then diluted at appropriate dilutions and were run in duplicate (TIMPs) or triplicate (MMPs). MMP to TIMP ratios were calculated for each group. Descriptive statistics were performed as well as a Mann Whitney test to determine differences between PTOA horses and controls. Spearman rank correlations were performed to determine correlations between MMPs and TIMPs. $P<0.05$ was considered significant.
Results: Concentrations for MMP-2, MMP-3, and TIMP-2 were all significantly higher in SF from PTOA joints compared to control joints ($P<0.05$). In addition, the MMP-2/TIMP-1, MMP-2/TIMP-4, and MMP-3/TIMP-3 ratios were all significantly higher in PTOA joints compared to control joints ($P<0.05$). In PTOA joints, 9/17 (53%) samples had MMP-3 concentrations higher than 100 pg/mL compared to only 3/14 (21%) for controls. In addition, in those PTOA horses in which MMP-3 concentrations were >100 pg/mL, 8/9 (89%) had MMP-1 concentrations >100 pg/mL, 7/9 (78%) had MMP-2 concentrations >0 pg/mL, 5/9 (56%) had MMP-9 concentrations >100 pg/mL, and 3/9 (33%) had MMP-13 concentrations >0 pg/mL, whereas control horses with MMP-3 concentrations >100 pg/mL had no horses with MMP-1 concentrations >100 pg/mL, MMP-2 concentrations >0 pg/mL, MMP-9 concentrations >100 pg/mL, and MMP-13 concentrations >0 pg/mL. MMP-1 and MMP-3 were highly correlated ($P<0.001$; $R = 0.793$) as was MMP-2 and TIMP-2 ($P = 0.002$; $R = 0.716$).
Conclusions: Post traumatic osteoarthritic joints have selectively higher MMP and TIMP concentrations than control joints. In addition, PTOA joints also have higher ratios of MMPs to TIMPs when examining MMP-2 relative to TIMP-1 and -4, as well as MMP-3 relative to TIMP-3. Most MMP concentrations were higher in PTOA joints than controls while TIMP concentrations generally stayed the same or decreased (except for TIMP-2). This indicates that when PTOA develops, TIMP concentrations generally do not directly increase in response to increased MMP concentrations. This could allow MMPs to further degrade the extracellular matrix of articular cartilage. TIMP-2 was the only TIMP to increase when PTOA was present, which may be related to its capability to drive the release and activation of MMP-2, as also demonstrated by the correlation between MMP-2 and TIMP-2. In those PTOA joints in which MMP-3 concentrations were >100 pg/mL, multiple other MMP concentrations were elevated as well whereas no control samples followed this pattern. This demonstrates the likely interplay between MMPs once PTOA develops where one MMP stimulates the production and the activation of others to drive further cartilage degradation. SF analysis of MMPs and TIMPs as indirect biomarkers of PTOA may prove to have value in monitoring the progression of OA, especially if combined with cytokines, chemokines, and traditional biochemical biomarkers of OA.

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ASSOCIATION BETWEEN BIOCHEMICAL CARTILAGE MARKERS AND CLINICAL SYMPTOMS IN PATIENTS WITH HIP OSTEOARTHRITIS: COHORT STUDY WITH TWO-YEAR FOLLOW-UP

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Purpose: Biomarkers are defined as characteristics that are objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. The availability of biomarkers that can assist in diagnosing early-stage OA, predicting OA progression, and assessing therapeutic responses could improve early diagnosis and help monitor the effect of OA treatment. The goal of this study was to assess the associations between two urinary biomarkers (CTX-II and CIIM) and severity of hip pain in patients with hip OA over a 2-year period, and establish whether the level of these biomarkers at baseline could predict a specific course of hip pain.
Methods: The study population consisted of primary care patients diagnosed with hip OA ($n = 222$) who participated in a prospective randomized controlled trial that assessed the effect of glucosamine sulfate (GOAL trial). Patients were eligible if they met one of the American College of Rheumatology criteria for hip OA. Patients who had undergone or were awaiting total hip replacement surgery and patients with a Kellgren and Lawrence (KL) score of 4 were excluded. Patients were also excluded if they had renal disease, liver disease, diabetes mellitus, or were already taking glucosamine. The outcomes were level of the biomarkers uCTX-II and uCIIM, and hip pain, measured with the Western Ontario and McMasters University Osteoarthritis Index (WOMAC) and with the Visual Analog Scale (VAS). All outcomes were measured at baseline, and at 6, 12, 18, and 24 months follow-up. Associations between level of and biomarkers and hip pain severity were assessed using linear mixed-model analysis for repeated measurements. Adjustments included: age, gender, body mass index, allocated treatment, duration of hip complaints, type of OA and severity of radiologic hip OA. A subgroup analysis was performed with patients with minimal radiologic hip OA (KL<2) and patients with definite radiologic hip OA (KL≥2). Using the 3-monthly repeated pain measurements during 2-year follow-up, five distinct hip pain courses (trajectories) were identified in the GOAL trial previously: mild pain, moderate pain, high pain, regularly progressive pain, and highly progressive pain. These trajectories of hip pain were used as outcome to investigate whether the level of biomarkers at baseline could predict membership in one of the five trajectories using multinomial regression analysis.
Results: The mean age of the 222 patients participating in the GOAL trial was 63.4 (SD 9.0) years and 69.4% of patients were female. At baseline, the mean WOMAC pain score was 34.2 (SD 23.1) and the mean VAS pain score was 32.4 (SD 25.9). The median uCIIM pg/umol creat was 61.7 (IQR 51.5) and the median uCTX-II pg/umol creat was 332 (IQR 355).
At baseline, biomarkers for 197 (89%) patients were available for analysis, compared with 177 (80%) patients at 6-months follow-up, 190 (86%) at 12 months, 186 (84%) at 18 months and 187 (84%) patients at 24-months follow-up.
Patients in the highly progressive pain trajectory and the moderate pain trajectory were more likely to have a higher loguCTX-II at baseline (OR 6.5; 95% CI 1.6-28.2 and OR 4.8; 95%CI 1.0-22.8, respectively) than patients in the mild pain trajectory.
Neither loguCTX-II nor loguCIIM were cross-sectionally associated with WOMAC pain or VAS pain during the 2-year follow-up. The subgroup analysis of patients with definite radiographic OA (K&L≥2) at baseline ($n = 108$) showed a significant association between loguCTX-II and VAS pain (coefficient 16.7; 95% CI 7.3-26.1). The cross-sectional association between loguCTX-II and WOMAC pain was not significant in the definite radiographic OA group, nor were the associations between loguCIIM and WOMAC pain or VAS pain. In the group with minimal radiographic OA at baseline ($n = 114$), no associations were found between loguCTX-II, loguCIIM and the pain scores.

Conclusions: This study shows that in patients with hip OA the urinary biochemical markers uCTX-II and uCIIM are not cross-sectionally associated with hip pain during the 2-year follow-up. However, the uCTX-II level at baseline could predict a progressive or moderate hip pain trajectory over a two-year follow-up period.

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EXTRACELLULAR MATRIX CHANGES IN RESPONSE TO RECOMBINANT HUMAN FIBROBLAST GROWTH FACTOR 18 STUDIED IN EX VIVO CULTURES OF ARTICULAR CARTILAGE

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Purpose: Osteoarthritis (OA) is a degenerative disease with high prevalence, creating an unmet medical need for drugs to regenerate cartilage. A promising candidate for a novel disease modifying osteoarthritis drug (DMOAD) is Sprifermin, a truncated form of fibroblast growth factor 18 (FGF18). Sprifermin has been demonstrated to increase cartilage volume in the knees of OA patients, but surprisingly little is known about the mode of action behind its anabolic effects. The few studies published indicate that full-length FGF18 induces cartilage formation by increasing chondrocyte proliferation, resulting in increased overall matrix production by the larger population of chondrocytes. Our hypothesis is that matrix degradation is initially needed during this process in order to expand the lacunae and make room in the matrix for the new chondrocytes. Accordingly, the aim of this study was to characterize the changes in matrix degradation occurring in response to direct stimulation with recombinant human (rh) FGF18.
Methods: Full depth cartilage explants (FDCex) punched from bovine articular cartilage were cultured for 27 days. In replicates of six, the FDCex were treated with various concentrations of full-length rhFGF18 (1, 10, 50, 100 or 500 ng/mL rhFGF18), an anabolic cytokine as positive control for cartilage formation (100 ng/mL IGF-I), or culture media without treatment as negative control (W/O). Supernatants were harvested and replaced 3 times weekly. Cell viability was measured using AlamarBlue at day 27. Biomarkers released to the supernatant were measured using the following well-described ELISA; C2M and AGNx2 reflecting matrix metalloproteinase (MMP)-mediated degradation of type II collagen and aggrecan, respectively, AGNx1 reflecting aggrecanase-mediated degradation of aggrecan, and C-Col10 reflecting chondrocyte hypertrophy. Mean values and standard error of the mean (SEM) were compared using one-way ANOVA assuming normal distribution. Significance levels are indicated by asterisks; $^{*}P < 0.05$, $^{**}P < 0.01$.
Results: To evaluate the changes in matrix degradation occurring in the FDCex in response to direct stimulation with rhFGF18, three different biomarkers of matrix degradation were quantified (figure 1). According to C2M, MMP-mediated type II collagen degradation is significantly decreased in response to $\geq$ 50 ng/mL rhFGF18 from day 11 onward ($P < 0.05$). Likewise, AGNx2 indicates a slight decrease in MMP-mediated aggrecan degradation in response to $\geq$ 50 ng/mL rhFGF18 from day 18 onward, although not significant. On the contrary, AGNx1 reveals increased aggrecanase-mediated aggrecan degradation in response to $\geq$ 10 ng/mL rhFGF18 at day 25, although only significant for 50 ng/mL rhFGF18 ($P < 0.05$). Evaluation of the chondrocytic changes occurring in response to rhFGF18, reveal a dose-dependent increase in cell viability measured at day 27, and no indication of hypertrophic cell differentiation, as assessed by C-Col10.
Conclusions: The data presented here indicate that direct stimulation of articular cartilage with rhFGF18 leads to decreased generation of MMP-mediated cleavage fragments of type II collagen and aggrecan, but increased generation of the aggrecanase-mediated cleavage fragment of aggrecan. Interestingly, AGNx2 is believed to be a marker of cartilage degradation with impaired repair capacity, whereas AGNx1 is considered a marker of reversible form cartilage degradation. Accordingly, the increased AGNx1 could indicate the matrix degradation needed for expansion of the lacunae, which according to our hypothesis is needed to initiate the process of cartilage formation. Clarifying the steps of this process is highly important for the understanding of how a potential novel DMOAD is affecting the tissue.

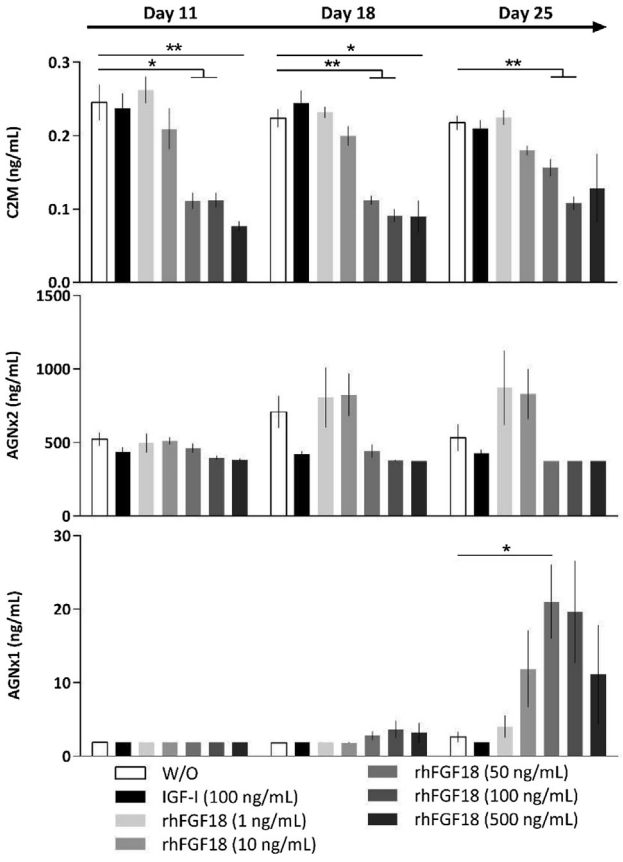

Figure 1. **Extracellular matrix degradation.** Bovine FDC explants were cultured with indicated treatments for 27 days. Supernatant were monitored by biomarkers of cartilage degradation C2M, AGNx1 and AGNx2 (Nordic Bioscience A/S). Values are mean ± SEM. One-way ANOVA was used to compare each bar with its respective W/O. $^{*}P < 0.05$, $^{**}P < 0.01$.

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URINE CROSSLINKED TELOPEPTIDE OF TYPE II COLLAGEN PREDICTED OSTEOARTHRITIS PROGRESSION

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Purpose: The gold standard for assessing joint damage is still the plain radiograph and this method only provides a historical view of the skeletal damage that has already occurred. We cannot predict OA progression from the radiograph. In contrast, biomarkers are candidates to predict an event that would occur in OA. One of the primary disease processes of osteoarthritis is degradation of the type II collagen, which is most abundant and highly specific for cartilage tissue. Crosslinked telopeptide of type II collagen (CTX-II) was one of the specific marker of type II collagen degradation in cartilage. Monitoring urine (u) CTX-II was considered to be a potential biomarker in knee OA. It is reported that uCTX-II was correlated with prevalence of radiographic OA and higher uCTX-II had a increasing risk of having OA. However, there were few reports about its role for prediction of OA progression. The purpose of this study was to investigate whether uCTX-II could predict OA progression.
Methods: This prospective cohort study protocol was approved by the institutional review board of our university. One hundred and fourteen painful medial knee OA patients were enrolled in this study. Some of the patients were excluded due to data and follow up missing. Ninety one patients (71.5y in average, men/female: 9/82) were analyzed. The basal characteristics of the patients and a standing, extended antero-